Monitoring of the
Lower Carson Slough Population of
Amargosa Niterwort
near Death Valley Junction, California
2010 – 2011

Bureau of Land Management
California State Office
Sacramento, California
January 17, 2012

Contents

Preface

The author of this report is John Willoughby, who designed and helped carry out the study pursuant to a contract with the Bureau of Land Management (BLM), California State Office. The contract was commissioned by Christina Lund, State Botanist, BLM California State Office, who conceived of the study and secured funding in support of it. Christina helped at all stages of the study, including measuring Amargosa niterwort plants in all of the 2011 transects. Karen Jones served as the data recorder in both 2010 and 2011. Naomi Fraga, Jeff Morawetz, Stephanie Rockwood, and Lindsay Ward of the Rancho Santa Ana Botanic Garden assisted by censusing Macroplot 4 in 2011. The study benefitted from discussions with Fred Edwards, currently botanist at the BLM Las Vegas District Office and formerly botanist with the Las Vegas Fish and Wildlife Office of the U.S. Fish and Wildlife Service (USFWS), and Brian Croft of the Ventura Fish and Wildlife Office of the USFWS. Russell Scofield, Department of the Interior Coordinator of the Desert Managers Group, supported this study from the beginning. He and Christina Lund also helped ensure the funding and implementation of an ongoing groundwater study in the Lower Carson Slough area which will facilitate interpretation of the information collected in this study.

Amargosa niterwort (*Nitrophila mohavensis*) was listed as Endangered by the U.S. Fish and Wildlife Service in 1985. The Five-Year Review of the species (USFWS 2007) concluded, based primarily on anecdotal information, the species has declined since its listing, presumably as a result of increased groundwater pumping. This study was designed to determine whether the species is in fact declining at its largest population, in the Lower Carson Slough near Death Valley Junction, California. The sampling objective of the study is to be able to detect differences of 30% (as a relative measurement) in estimated Amargosa niterwort parameters with a false-change (Type I) error rate of 0.10 and a missed-change (Type II) error rate of 0.10. Because Amargosa niterwort is a rhizomatous perennial species, it was decided to use permanent belt transects to reduce the effects of spatial variability on the study. The sampling units (belt transects) are thus paired with one another over time. Because sample size determination in a paired-sample design requires an estimate of the standard error of the differences between sampling units in at least two years, this two year study essentially served as a pilot study to inform the longer term monitoring of this species.

Executive Summary

Amargosa niterwort (*Nitrophila mohavensis*) at the Lower Carson Slough near Death Valley Junction, California, was monitored in 2010 and 2011. Five macroplots were established in an area recognized as supporting most of the niterwort plants in the Lower Carson Slough population. Because Amargosa niterwort is a rhizomatous species, it is not possible to count individual plants. Therefore, the first year of monitoring involved counting the number of rooted stems and clumps of stems. Estimates of the number of rooted stems were available from a previous study conducted in 2003. Because of severe problems involved in counting rooted stems without damaging plants, the number of clumps of stems was added as a counting unit (clumps are single plants or groups of plants separated by at least 2 cm from each other from the place they are rooted). Based on the experience of the 2010 monitoring, another parameter, cover, was estimated beginning in 2011.

A complete census was conducted in Macroplot 4. The other four macroplots (1, 2, 3, and 5) were sampled by means of belt transects that were either 0.5 m x 75 m (macroplots 1, 2, and 5) or 0.5 m x 100 m (Macroplot 3) in size. Counts and cover measurements were recorded in 1 m segments along each of the belt transects. Counts were recorded in 1 m segments along each of the belt transects. This allows for analysis using total belt transect values (by summing segment values along each transect), and also makes it possible to estimate the frequency of occurrence in the 1m segments. Taking data by transect segment also provides information on the spatial distribution of the species that would not be possible if only transect totals were recorded.

A total of 23 belt transects were sampled in 2010 and permanently marked. These same 23 belt transects were sampled in 2011, and—based on a sample size analysis—an additional 7 belt transects were permanently marked and read in 2011.

The estimates for the number of rooted stems in the combined area of Macroplots 1-5 were 59,540 (± 24,782) in 2010 and 58,431 (± 21,541) in 2011 (± 95% confidence interval). The slightly lower estimate for 2011 was not statistically significant. Because of problems inherent in accurately counting rooted stems (see body of paper for details), these numbers are not considered to accurately track niterwort abundance between years. In fact, rooted stems will be dropped as a measured attribute in future years.

Both the estimated number of clumps and frequency were greater in 2011 than in 2010. Clump numbers were estimated to be 33,309 (± 12,895) in 2011, about twice as many as estimated for 2010 (16,712 ± 5,938), a difference that was statistically significant. The 2011 frequency of

0.088 (± 0.027) was significantly greater than the 2010 frequency of 0.061 (± 0.015). Although total growing season precipitation was about the same for the two years, the higher niterwort abundance observed in 2011 appears to result from a better distribution of precipitation during the hotter months, an observation that is supported by groundwater levels measured in a piezometer immediately adjacent to the niterwort population at Lower Carson Slough.

Although problems with the 2003 study preclude quantitative comparisons between stem number estimates from 2003 to those from 2010-2011, it seems likely that niterwort abundance as inferred from stem numbers was considerably lower in 2010-2011 than in 2003. This appears to be the result of both higher growing season precipitation in the 2002-2003 growing season (4.78 in.) compared to the amount of precipitation in the 2009-2010 and 2010-2011 growing seasons (3.61 in. and 3.67 in., respectively) and much higher precipitation than normal in the hotter months of April, May, and July 2003.

Based on this study, the 2003 study, and a 2005-2006 study of the water use of Amargosa niterwort, there appears to be considerable year-to-year variability in niterwort abundance. This will make it more difficult to tease out the effects of increased groundwater pumping from natural variability resulting from differences in the amount and timing of precipitation, something that will only be possible if this study is continued yearly for at least several more years.

Introduction

Amargosa niterwort (*Nitrophila mohavensis*), a member of the plant family Chenopodiaceae, is known from only three occurrences in southwestern Nevada and southeastern California: the Ash Meadows National Wildlife Refuge in Nevada, the Lower Carson Slough area downstream from Ash Meadows in California (near Death Valley Junction), and a site near Tecopa Hot Springs in California. Because of its rarity and dependency on ground water, the species was listed as Endangered by the State of California in 1979 and as Endangered by the U.S. Fish and Wildlife Service in 1985. The Amargosa niterwort occurrence in the Lower Carson Slough area is on public lands under the jurisdiction of the Bureau of Land Management's Bartow Field Office and is the target of this monitoring plan. The Lower Carson Slough occurrence of Amargosa niterwort was the target of a previous inventory effort conducted in 2003 by Anteon Corporation under contract with BLM (Johnston and Zink 2004).

The Five-Year Review of Amargosa niterwort (USFWS 2007) concluded, based principally on anecdotal information, the species has declined since its 1985 listing, presumably because of declines in groundwater from increased pumping. Based on visits to the Lower Carson Slough following the 2003 Anteon study, Fred Edwards, currently the botanist for the BLM Las Vegas District and formerly botanist with the Fish and Wildlife Service's Las Vegas Field Office and author of the Five-Year Review, believes Amargosa niterwort has declined since the 2003 study (F. Edwards, personal communication 2010).

Initially it was hoped that it might prove possible to essentially continue the methodology used in the Anteon inventory effort in the monitoring set to begin in 2010, which would then allow direct comparisons of estimates from 2010 and subsequent monitoring to the 2003 estimates and allow conclusions to be made as to whether the species has in fact declined since 2003. Unfortunately, because of problems with the Anteon study design and the inability of BLM to obtain the raw data from that inventory, this was not possible. Because Anteon's estimates are of questionable validity, it is not even possible to quantitatively compare the 2010 and 2011 estimates to Anteon's estimates. A summary of the Anteon study and its problems is given in Appendix 1.

Objective of this Monitoring Study

Given the inability to design a monitoring plan that would enable a quantitative comparison between newly collected 2010 data and the summary statistics provided for the 2003 Anteon data, the focus of the 2010 study shifted to a design that would allow detection of future changes in the abundance of Amargosa niterwort. Because Occurrence 1 as delineated in the 2003 Anteon study contained a much greater number of plants than Occurrence 2 and therefore represents more optimum habitat for the species, it was decided to focus the monitoring effort on the area within Occurrence 1. Although it might seem important to also monitor the species in Occurrence 2, the sparseness of the species in that area precludes adequate sampling within current budgets. The only map available depicting Occurrence 1 is the 8.5 in. x 11 in. map in the Anteon report reproduced here as Figure 1. Occurrence 1 from this low resolution map was digitized into a Geographical Information System (ArcGIS 9.2, ESRI 2009) and six macroplots of varying dimensions were superimposed on this GIS map (Figure 2). These macroplots were to function as strata in a stratified random sampling design and would be used to facilitate the random placement of long, narrow quadrats (also called belt transects; Elzinga et al., 1998 and 2001).

Because Amargosa niterwort is a rhizomatous perennial species (Figure 3), it was decided to use permanent belt transects to reduce the effects of spatial variability on the study. The sampling units (belt transects) are thus paired with one another over time. Because sample size determination in a paired-sample design requires an estimate of the standard error of the differences between sampling units in at least two years, this two year study essentially served as a pilot study to inform the longer term monitoring of this species.

The initial plan was to distribute 28 belt transects, each 0.5m wide, among the 6 macroplots as shown in Figure 4. The transects would be positioned perpendicular to the baselines indicated in Figure 4 using a restricted random design (described in more detail below). Because of the rhizomatous growth habit of the species, it is not possible to delineate and count individual plants (genets). Therefore, the measurement unit would be rooted stems as defined by Anteon (more on this below).

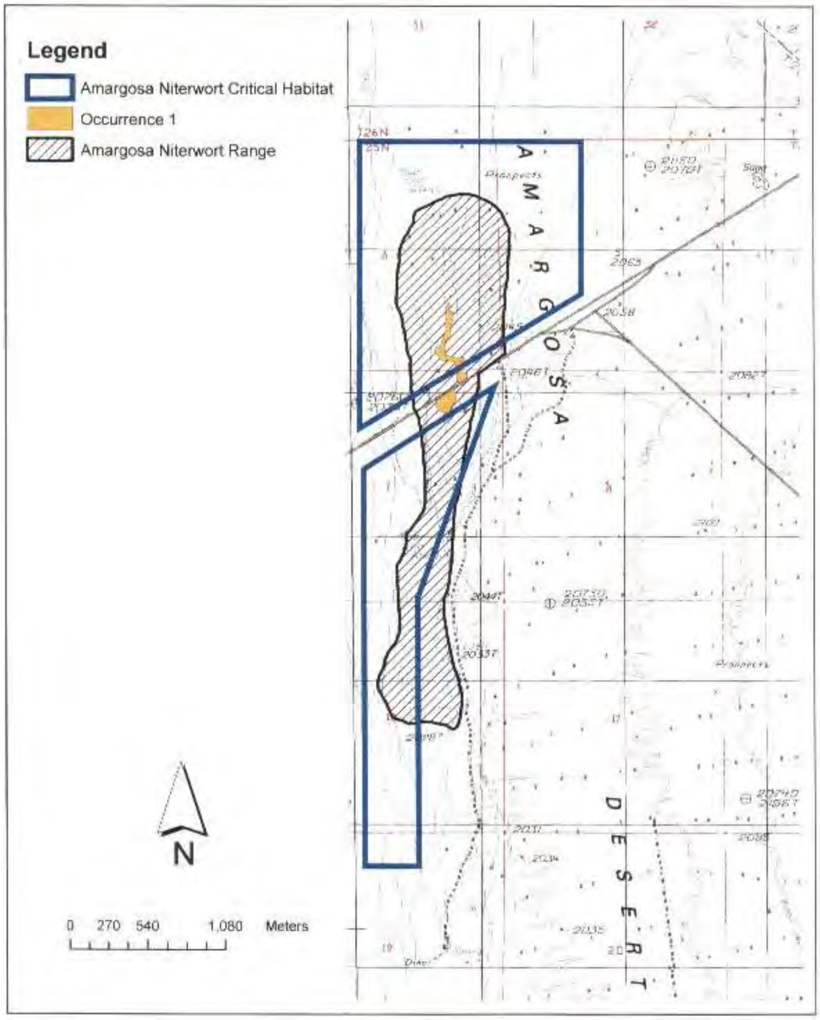


Figure 1. Map from Thomas and Zink 2004, showing Amargosa niterwort Occurrence 1. Although not explicitly stated in the report, the crosshatched area on the map (labeled "Amargosa Niterwort Range") minus the Occurrence 1 area is presumably their Occurrence 2.

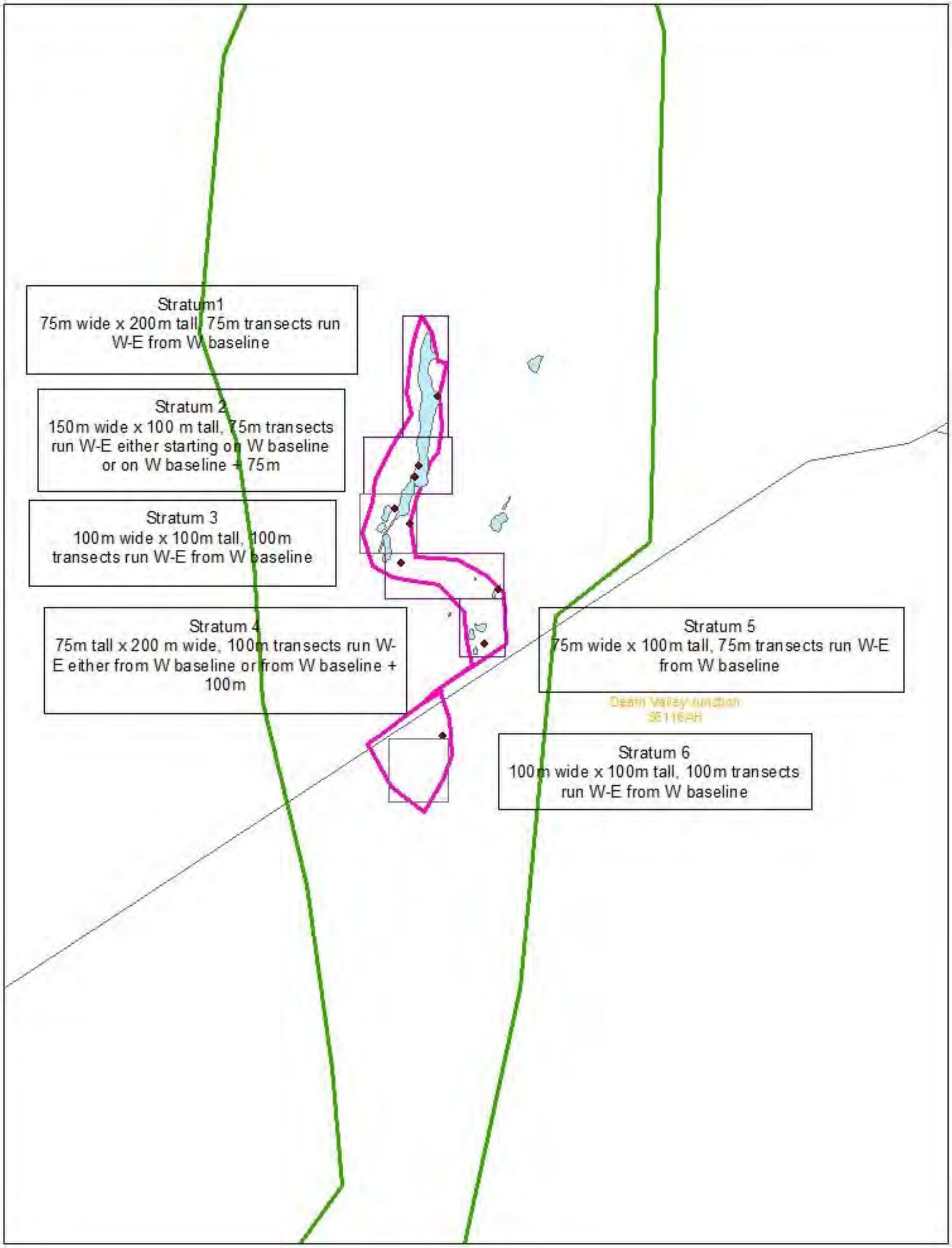


Figure 2. Original plan: 6 macroplots (strata) covering most of Thomas and Zink (2004) Occurrence 1.

Figure 3. Amargosa niterwort rhizomes excavated from the population at Ash Meadows National Wildlife Refuge below Crystal Reservoir. Photograph by Sara Scoles-Sciulla and Emily Beamguard, U.S. Geological Survey, Henderson, NV.

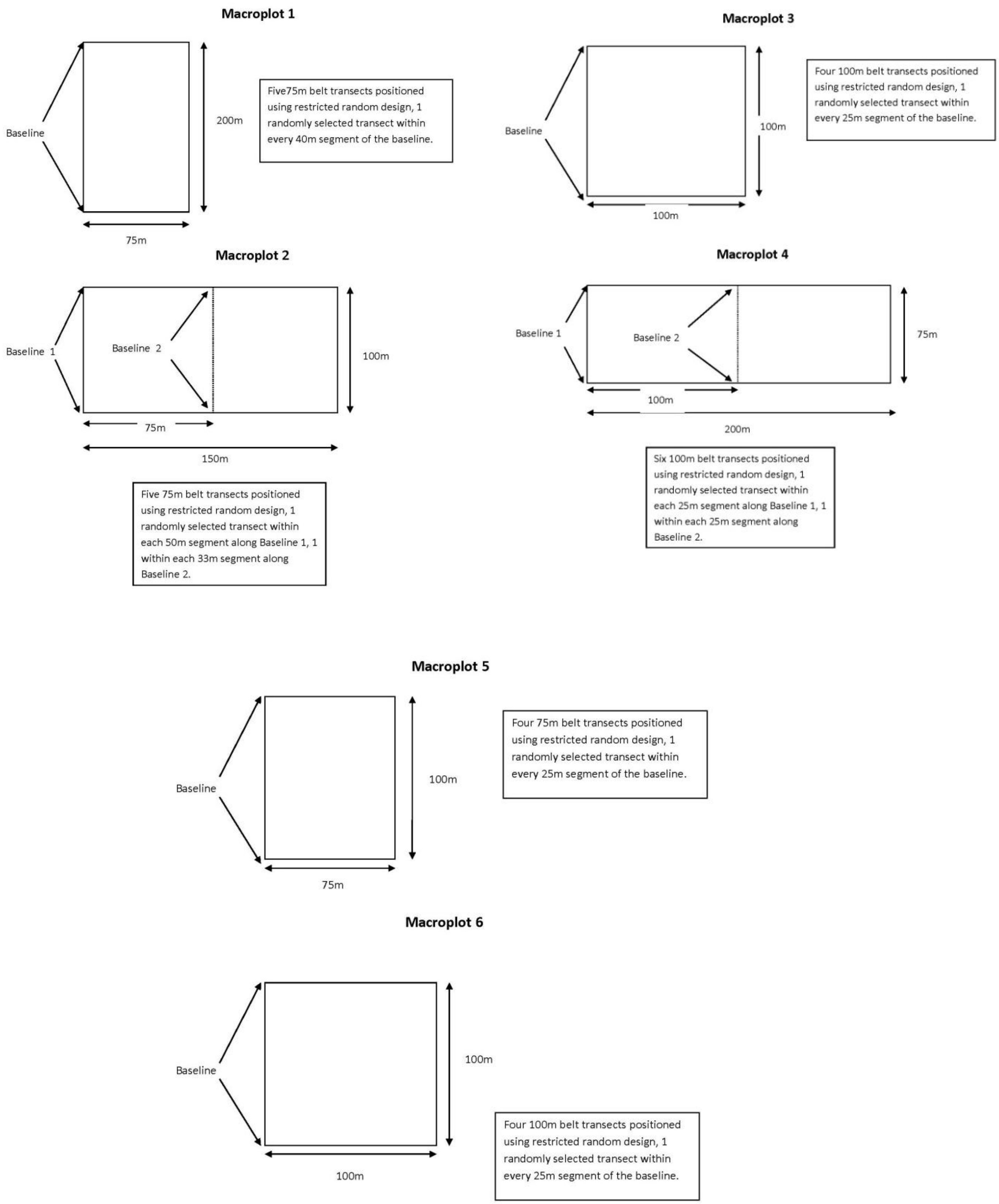


Figure 4. Original design of 6 macroplots and number of belt transects to be positioned in each.

Sampling Objective

Although the data collected from the study can (and will) be used to estimate the total number of stems within the area sampled in each year, the principal objective of the monitoring is to detect changes in Amargosa niterwort abundance[1] between years. The sampling objective is therefore framed as a change/trend objective as defined in Elzinga et al. (1998 and 2011):

> Be able to detect a 30 percent change in the number of stems of Amargosa niterwort between any two years in the area within the collection of 6 macroplots with a false-change (Type I) error rate of 0.10 and a missed-change (Type II) error rate of 0.10.[2]

Because the sample size necessary to meet the sampling objective depends upon the standard deviation of the population being sampled, the sample size could not be calculated before the population was actually sampled. Further, because this monitoring study uses permanent belt transects, the standard deviation necessary to calculate the sample size is the standard deviation of the collection of *differences* between each permanent belt transect at time 1 and time 2. Thus, two years of pilot sampling are required in order to calculate sample size. Year 1 sample size is basically just an educated guess at the number of transects that may be required. Once both Year 1 and Year 2 data have been collected, the number of transects can be revised to ensure the sampling objective will be met. However, because the transects are paired, if more transects are necessary to meet the objective, these transects will be added in Year 2, meaning that only differences between Year 2 and future years will have the required power to detect the level of change in the sampling objective (although changes between Year 2 and Year 1 can still be tested at a lower level of power).

2010 Monitoring and Changes to the Initial Study Design

Actual monitoring began on September 10, 2010. Field reconnaissance showed that the initial positioning of the 6 macroplots was not going to work as planned because the western parts of several of these macroplots were not habitat for the species and the macroplots would fail to include many plants observed to the east of the macroplot boundaries. This may be a function of the inevitable error associated with digitizing from an 8½ x 11 in. map. It may also at least partially result from Anteon mapping areas within Occurrence 1 that were not really occupied by plants. Anteon's sampled plots were all in the eastern part of Occurrence 1 because of

[1] "Abundance" is used here as a generic term and refers to such above-ground attributes as number of rooted stems (the initial metric), number of clumps, percent cover, and frequency. As the discussion section will point out later in the report, real abundance is likely better correlated with the parts of Amargosa niterwort that are below ground: rhizomes and seeds.

[2] Because statistical power is the complement of the missed-change error rate, the power of this design to detect a change of 30% would be 0.90 (or 90% expressed as a percentage).

apparent bias in the sampling of Occurrence 1 (see Appendix 1). It was the western part of the proposed macroplots that were devoid of plants.

Whatever the reason for the discrepancy, the macroplots were redefined in the field. The number of macroplots was reduced from six to five. One macroplot, Macroplot 4, was positioned to encompass a particularly dense population of Amargosa niterwort near Stateline Road. Its small size (20m x 30m) enables observers to completely census it, allowing for more precise tracking of niterwort abundance than can be accomplished in the other larger macroplots. The other four macroplots are larger and positioned in areas that are representative of Anteon's Occurrence 1. Figure 5 shows the location of these macroplots and the 23 transects located within them. Macroplot size, number of transects, and length of transects are given in Table 1.

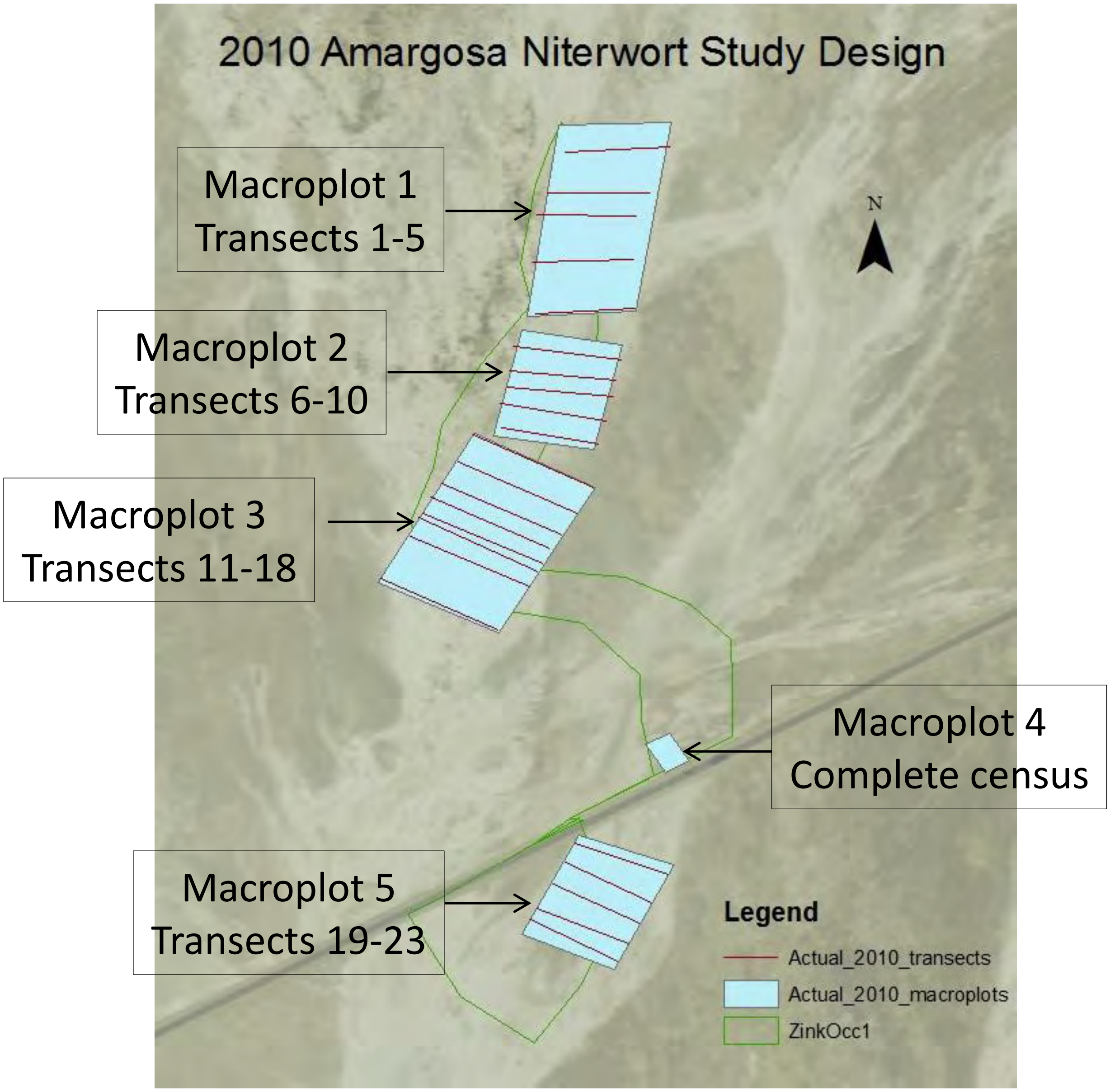


Figure 5. Monitoring design actually implemented in the field in 2010. Five macroplots were established. The smallest one of these, Macroplot 4,was completely censused for the number of rooted stems and clumps. The other four were sampled by means of belt transects, 0.5m wide and either 75m or 100m long, depending on the width of the macroplot.

Table 1. Macroplot size, number of transects in 2010, transect length, and location of intermediate markers (12 in. nails) along each transect. All transects are 0.5m wide.

Macroplot Number	Macroplot Size (m) Baseline Length Given First	Number of Transects in 2010	Transect Length (m)	Points (m) along transects at which intermediate markers (12 in. nails) are installed
1	200 x 75	5	75	15, 30, 45, 60
2	100 x 75	5	75	15, 30, 45, 60
3	150 x 100	8	100	15, 30, 45, 60, 75, 90
4	30 x 20	*	*	*
5	100 x 75	5	75	10, 20, 30, 40, 50, 60, 70

* Macroplot 4 was completely censused.

Transects were positioned using a restricted random sampling design. This entails dividing the baseline of each macroplot into segments corresponding to the number of transects allocated to each macroplot. For example, the baseline of Macroplot 1 is 200m long with 5 transects allocated to that macroplot. The baseline is therefore divided into 5 segments, each 40m long, and a single transect is randomly positioned in each segment. For the first segment, a random number between 0 and 39 is selected, with the selected number corresponding to the position on the baseline from which transect 1 will be run perpendicular to the baseline. A random number is then selected between 40 and 79 to position transect 2, and so on. Transect lengths are either 75m (for macroplots 1, 2, and 5) or 100m (for Macroplot 3).

Each transect was given a unique number. The five transects in Macroplot 1 were numbered 1-5, the five in Macroplot 2 were numbered 6-10, the eight in Macroplot 3 were numbered 11-18, and the five in Macroplot 5 were numbered 19-23 (no transects were used in Macroplot 4 because that macroplot was completely censused). Geographic coordinates for transect starting and ending points are given in Appendix 2; those for macroplot locations are given in Appendix 3.

Monitoring was conducted September 10-14, 2010, with John Willoughby doing the measurements (counts) and Karen Jones doing the recording. Originally only rooted stems were going to be counted, using the same measurement unit as used in the Anteon study.[3]

It quickly became apparent, however, that a rooted stem is a poor measurement unit. Where niterwort stems are solitary or few there is no problem counting rooted stems (Figure 6a). But where niterwort stems are very clumped it is impossible to accurately count rooted stems without damaging plants (Figure 6b). Because the observers (at least in 2010 and again in 2011) took great care not to damage plants, stem counts are more ocular estimates than actual counts. This is unacceptable because it results in a high level of non-sampling error which cannot be quantified. Estimates of population size will be biased, probably on the low side, and conclusions as to whether there has or hasn't been a change in stem numbers cannot be trusted. Because of this, the decision was made to add a second measurement unit that could be more accurately counted. This second measurement unit is the number of clumps. Clumps are single plants or groups of plants separated by at least 2 cm from each other from the place they are rooted.

Rooted stems and clumps were counted in 0.5 m x 75 m or 0.5 m x 100 m belt transects (long, narrow quadrats). Transects were run perpendicular from the randomly selected positions along baselines set up on the west side of each macroplot. The north end of the baseline is the 0 point of the baseline. One long side of each belt transect (the northern side or left side as viewed from the baseline) was laid out using a fiberglass tape measure. This long side was then anchored at each end with pieces of aluminum conduit about 2.5 ft. (76 cm) long, pounded about 1 ft. (30 cm) into the ground. The beginning and ending points of each transect were labeled by means of aluminum labels (IMPRES-O-TAGS, Amekron Products) that were marked with the transect number, date of establishment, and whether the point represents the beginning or ending point of the transect. Tags were affixed to the aluminum conduit using wire loops. In addition, intermediate points along transects were marked with galvanized 12 inch (30 cm) nails driven nearly to the ground at 15 m or shorter intervals to ensure that tapes will be laid out in the same position each year. The fiberglass tape is anchored at each end by the aluminum conduit. The tape is then anchored to each intermediate nail by means of a large binder clip. See Table 1 for the intermediate point locations along transects within each of the

[3] As described in Appendix 1, the Anteon study defined the counting unit as follows: "A count of one ramet consisted of a single stem arising from the soil." For simplicity, such stems are called "rooted stems" in this report to distinguish them from stems that branch above the ground level (the latter stems are not counted; i.e., a stem arising from the ground with several stems branching from it above the ground would result in a count of 1).

a.

b.

Figure 6. Problems in counting individual rooted stems. Where stems are solitary (a) there is no difficulty in counting the number of rooted stems, but where stems are highly clumped (b) it is impossible to accurately count the number of rooted stems without damaging the plants.

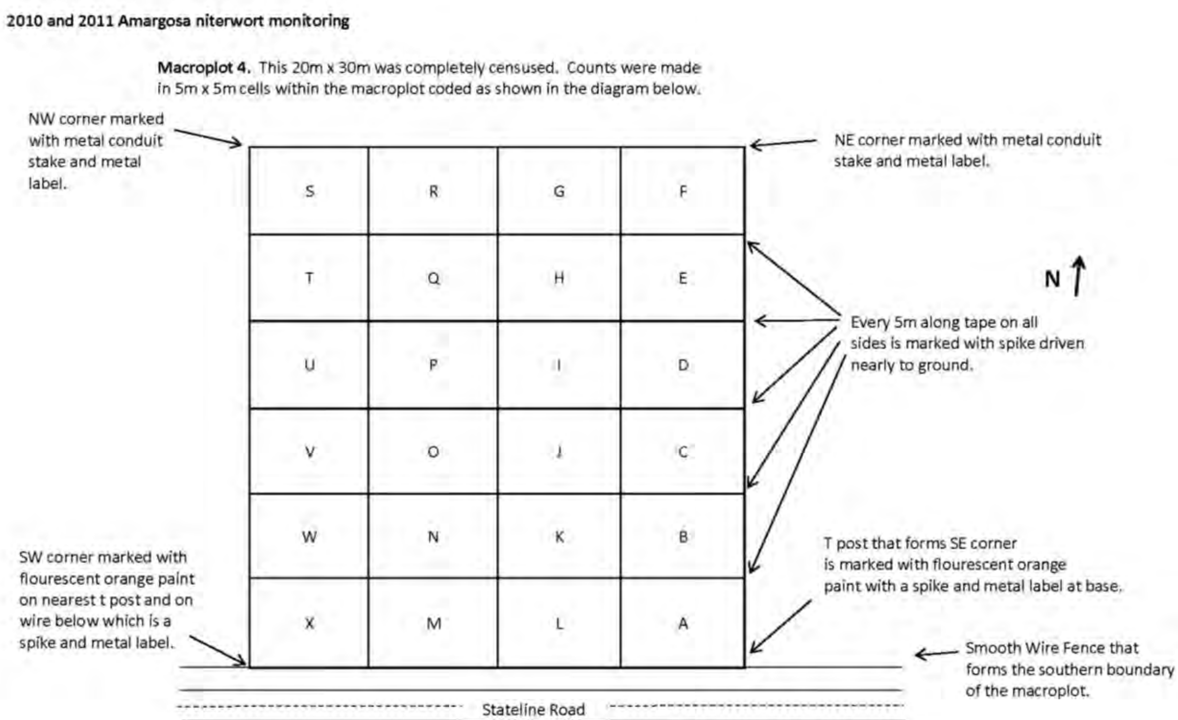
macroplots. To insure against potential loss of the aluminum conduit at each end of the transect, 12 inch (30 cm) nails were placed 1 m beyond each end of the transect (in line with the transect) to facilitate replacement of conduit at transect starting and ending points. The only exceptions to this 1 m distance were transects 6 and 8 where shrubs precluded the nails being placed 1 m beyond the start of each of these transects. Appendix 2 shows the positions of the nails beyond the starting points of transects 6 and 8. Once the tape measure corresponding to the northern (left) side of the belt transect was positioned and secured to the conduit and intermediate nails, a 0.5 m piece of PVC was placed perpendicular to the tape to determine where the south (right) side of each belt would fall. It is only necessary to use the piece of PVC at points along the transect where niterwort plants occur that might fall inside the belt.

Counts were recorded in 1 m segments along each of the belt transects. This allows for analysis using total belt transect values (by summing segment values along each transect), and also makes it possible to estimate the frequency of occurrence in the 1m segments. Taking data by transect segment also provides information on the spatial distribution of the species that would not be possible if only transect totals were recorded.

To minimize any edge effects associated with the use of long belt transects, the following rules were employed (and it will be important to adhere to these rules in future monitoring). Stems or clumps whose rooted stems fall on the left side of the belt are counted as in; those that fall on the right side are out. Stems or clumps whose rooted stems fall on the bottom edge of each 1 m segment are recorded for that segment. Those that fall on the top edge of each 1 m segment are out (but would be recorded in the next segment). Those that fall on the top edge of the last segment are not recorded.

Macroplot 4 was completely censused by dividing the 20 m x 30 m macroplot into twenty-four 5 m x 5 m cells and counting and recording stems and clumps separately for each cell. Macroplot totals were then derived by simply adding the counts for each cell. Figure 7 shows how the cells were laid out. Lengths of aluminum conduit, approximately 2.5 ft. (76 cm) long, were pounded approximately 1 ft. (30 cm) into the ground at all four corners of the macroplot. These corners were labeled by means of aluminum labels (IMPRES-O-TAGS, Amekron Products) that identify the macroplot and corner. Tags were affixed to the aluminum conduit using wire loops. Nails 12 in. (30 cm) long were placed at 5 m intervals around the perimeter of the macroplot. The southern boundary of the macroplot is immediately adjacent and parallel with the smooth wire fence that borders Stateline Road. The fence was marked with florescent orange paint at points corresponding to the SE and SW corners of the macroplot. Measuring tapes were used to circumscribe the perimeter of the macroplot. Measuring tapes were then attached to each of the nails in a criss-cross pattern to delineate the cells shown in Figure 7 (string or narrow diameter rope could be used in future monitoring in lieu of measuring tapes). The baseline is the eastern side of the macroplot, with the SE corner of the macroplot functioning as the 0 m point on the baseline. Geographic coordinates for each corner (NAD 83) are given in Figure 7.

Appendix 4 shows the data form used in 2010 for reading the 23 transects in macroplots 1, 2, 3, and 5. The decision to establish and completely census Macroplot 4 was made while in the field



Coordinates for the macroplot corners are as follows:

Corner	Latitude (NAD 83)	Longitude (NAD 83)
NE	36.32612450	-116.36734849
SE	36.32589751	-116.36719000
NW	36.32603813	-116.36754446
SW	36.32580405	-116.36737715

Figure 7. Macroplot 4 layout. The eastern boundary of the macroplot functions as the baseline, with the 0 m point starting on the southeast corner. Each 5 m x 5 m cell is identified by a letter as shown. Each cell is completely censused for number of rooted stems and counting clumps in 2010 and 2011, and for the area of cover clumps in 2011 and in future years. After 2011 rooted stems will no longer be censused. The census values for the cells are added together to derive the census total for the entire macroplot. Geographic coordinates (NAD 83) are given in decimal degrees latitude and longitude.

in 2010. Therefore, no monitoring form was prepared prior to monitoring and data were collected on blank sheets of paper. The methodology used, however, is reflected in the Macroplot data form prepared for the 2011 monitoring that is included in Appendix 5.

The sampling objective is essentially the same as the one developed before 2010 field work began, except that it was changed to account for the use of 5 rather than 6 macroplots and to focus on clumps rather than stems as clumps are more accurately counted.

Revised sampling objective:

> Be able to detect a 30 percent change in the number of clumps of Amargosa niterwort between any two years in the area within the collection of 5 macroplots with a false-change (Type I) error rate of 0.10 and a missed-change (Type II) error rate of 0.10.

2011 Monitoring and Further Changes to the Study Design

The second year of monitoring was conducted August 25-29, 2011. Christina Lund did most of the measurements (counts and cover measurements—see below) and Karen Jones was the recorder. John Willoughby did a few of the measurements and helped with other aspects of the monitoring including transect positioning and data analysis. The census of Macroplot 4 was conducted by the following personnel from Rancho Santa Ana Botanic Garden: Naomi Fraga, Jeff Morawetz, Stephanie Rockwood, and Lindsay Ward.

Rooted stems and clumps were counted in the same 23 transects that were read and permanently marked in 2010. As in 2010, counts were made in 1 m segments along each transect. Beginning in 2011, cover measurements of Amargosa niterwort were also made. This was accomplished by measuring clumps in two dimensions, along the x axis parallel to one edge of the belt transect and the y axis parallel to the other perpendicular edge of the belt. It didn't matter which dimension was reported as the x and which the y, as long as the measurements were parallel to the two perpendicular edges of the belt. The dimensions corresponding to the longest extent of each axis covered by the plant were recorded. Measurements were made to the closest 0.5 cm. A clump for cover measurements ("cover clump") is defined as a plant or group of plants with less than a 2 cm gap *in canopy cover* along the two long dimensions measured.[4] Area covered is calculated by the formula for the area of an ellipse:

[4] Note that a cover clump is not the same as a counting clump. A clump for counting purposes ("counting clump") is a single plant or group of plants separated by at least 2 cm from another plant or group from the place they are rooted. A clump for cover measurements ("cover clump") is defined as a plant or group of plants with less than a 2 cm gap in canopy cover along the two long dimensions measured.

$$\text{Area} = \pi ab$$

Where: $\pi = pi = 3.1416$

$a = \frac{1}{2}\, x_{dimension}$

$b = \frac{1}{2}\, y_{dimension}$

Cover calculated in this way will result in an overestimate of the actual foliar cover of Amargosa niterwort, because some area within each ellipse will not be covered by actual niterwort foliage. But a consistent application of this methodology will allow for detection of canopy cover changes over time.

After the 23 transects were monitored in 2011, the data were plugged into a sample size formula (DSTPLAN 4.2, Brown et al. 2000). Because of the problems involved in counting individual rooted stems, it was decided to dispense with individual stems as a counting unit. Therefore, only the number of clumps was used to determine the sample size necessary to meet the sampling objective. Based on an analysis conducted while in the field, it was determined that a sample size of 32 transects would meet the sampling objective. After looking at the area represented by each transect in each macroplot, it was decided to add 5 additional transects to Macroplot 1 and 2 additional transects to Macroplot 3 in order to equalize the area represented by each transect. After adding these 7 transects, the area represented by each transect in the four sampled macroplots is 1500 m^2. Although the new total of 30 transects will not quite meet the sampling objective, it was decided to stick with the 30 transects to keep the allocation proportional to area, at least for another year. The 30 transects will have a power of 0.88 to detect a change of 30 percent in the number of clumps, with a false-change error rate of 0.10. Looked at another way, this design will be able to detect a change of 31 percent in the number of clumps with power of 0.90 and false-change error rate of 0.10.

The 7 additional transects were added using a restricted random sampling design. The 5 additional transects added to Macroplot 1 were positioned by randomly selecting starting points in each of the 40 m segments used to site the original 5 transects. Had any of the random starting points selected for the additional transects been the same as the starting points used for the original transects, the duplicate point would have been rejected and another point chosen using the same procedure. As it turned out, this did not occur, and all 5 of the additional starting points from the initial random draw were retained. Starting points for the 2 additional transects added to Macroplot 3 were randomly selected in each of two 75 m segments of the 150 m baseline, again with the provision that if the starting points for any of these additional transects was the same as any of the starting points for the original transects, the duplicate point would be rejected and another point selected. This did not occur so the first two randomly selected starting points for the additional transects were used.

Rooted stems were not counted in the 7 transects added in 2011 since it had already been determined that this measurement is not accurate enough to continue to use. Only counting clumps and cover clumps were measured, as these will be the only attributes measured from 2012 onward (frequency will also be used but this attribute is a natural byproduct of the clump and cover sampling—see below). Appendix 5 shows the data forms used in 2011.

Data Analysis and Results

Data were analyzed using the survey procedures in Stata 10.0 (StataCorp 2007), which allow for the proper analysis of stratified random sampling designs. In addition to calculating estimates and 95 percent confidence intervals for rooted stems, clumps, and percent cover, estimates were also made of the frequency of the 0.5 m x 1.0 m quadrats used along the transects to derive transect totals of stems, clumps, and percent cover. Because these quadrats are not independent sampling units, transect frequencies were used in the analysis. These were derived by dividing the number of quadrats along a particular transect that included any Amargosa niterwort plants ("hits") by the total number of quadrats along that transect. Means and 95 percent confidence intervals were then calculated using Stata's survey procedures (the "svy: mean" command). Percent cover was calculated for each transect by summing the covered areas measured for each niterwort shoot or clump of shoots for the entire transect and then dividing that total cover by the total area of the transect. Estimates of total cover were then obtained using the "svy: mean" command in Stata.

Estimates of total numbers of rooted stems and clumps were obtained by using the "svy: total" command in Stata. Estimates of the mean number of rooted stems and clumps per square meter were obtained by using the "svy: ratio" command in Stata.[5]

Table 2 gives estimates for total rooted stems and clumps, number of rooted stems and clumps per square meter, and percent cover for the total area within the collection of five macroplots. It also gives estimated frequencies of niterwort occurrence within 0.5 m x 1.0 m quadrats for the total area within the combined macroplots 1, 2, 3, and 5 (because quadrats were not used

[5] Belt transects of different areas can sometimes be problematic when calculating means and totals. For example, Stehman and Salzer (2000) recommend that ratio estimation be used in lieu of simple mean calculations when belt transects have different areas. Because this study includes belt transects of different areas, it would at first appear that ratio estimation should be used instead of mean estimation (the "svy: total" command in Stata essentially estimates mean values and then converts those values to totals), such is not the case because the transect areas are the same within strata, and Stata controls for transect area differences between strata in its survey estimation procedures. To test this, Stata's "svy: ratio" command was used to derive estimates of the number of rooted stems and clumps per square meter (this is a ratio estimation procedure). When those ratio estimates were converted to population totals by multiplying the per square meter values by the total area within the collection of macroplots (45,600 m^2), the resulting values (totals and 95 percent confidence intervals) were exactly the same as those obtained using Stata's "svy: total" command.

to census Amargosa niterwort in macroplot 4 and frequency is a relative measurement requiring estimation in the same quadrat size and shape, no frequency estimates were possible for Macroplot 4). The precision for each estimate shown in Table 1 is calculated by dividing the confidence interval width (also shown in Table 1) by the estimate and multiplying by 100 to express the precision as a percent value. The smaller the precision value the better the estimate.

Table 2. Estimates, 95% confidence intervals, and precisions for Amargosa niterwort parameters estimated in 2010 and 2011.

	2010			2011		
Parameter	Estimate	95% Confi-dence Interval (±)	Precision of Estimate (%)	Estimate	95% Confi-dence Interval (±)	Precision of Estimate (%)
Total Number of Rooted Stems	59,540	24,782	41.62	58,431	21,541	36.87
Number of Rooted Stems/m^2	1.306	0.543	41.62	1.281	0.472	36.87
Total Number of Clumps	16,712	5,938	36	33,309	12,895	38.71
Number of Clumps/m^2	0.366	0.130	36	0.730	0.283	38.71
Cover	*	*	*	0.129%	0.055%	42.43
Frequency	0.061	0.015	25	0.088	.027	31.22

* Cover was not measured in 2010.

Differences between years were tested using paired *t* tests. These tests were run using the lincom command in Stata 10 following each survey estimation procedure. This post-estimation command (which stands for “linear combinations of estimators”) uses the appropriate standard errors derived from Stata’s stratified random survey procedures. Table 3 gives the results of these tests.

Table 3. Results of paired-sample *t* tests comparing estimated Amargosa niterwort parameters for 2010 and 2011. Because percent cover was not measured in 2010, no comparison of the change in that parameter is possible. *P* values < 0.10 are significant and are marked with asterisks. The *t* and *P* values are the same for total rooted stems and rooted stems/m^2 and for total clumps and clumps/m^2 because each pair of estimates uses the same data set. *P* values marked with asterisks were significant at $\alpha = 0.10$.

Parameter	2010 Estimate	2011 Estimate	Difference 2011-2010	Standard Error	*t* value	*P* value
Total Rooted Stems	59,540	58,431	-1,109	8,798	-0.13	0.900
Rooted Stems/m^2	1.306	1.281	-0.024	0.193	-0.13	0.900
Total Clumps	16,712	28,069	11,358	3,344	3.40	0.002*
Clumps/m^2	0.366	0.616	0.249	0.073	3.40	0.002*
Frequency	0.061	0.078	0.017	0.007	2.31	0.032*

Weather and Groundwater Information

Weather data were obtained from the Community Environmental Monitoring Program (CEMP) weather station at Amargosa Valley, Nevada (WRCC 2011). This station is the closest station to the monitoring site at Carson Slough, located about 28.0 km NNW of the monitoring site at an elevation of 2424 ft. (the elevation of the monitoring site is about 2065 ft.). The station began recording on September, 1999, so data are available for the 2002-2003 growing season (the growing season of the Anteon monitoring) as well as for the 2009-2010 and 2010-2011 growing seasons during which the two years of monitoring reported in this document were collected. There is another CEMP weather station at Tecopa, California, but this station is farther away from the monitoring site (54.9 km SSE from the monitoring site), at a lower elevation 1337 ft.), and did not start recording until February 2006, meaning that no data are available for the 2002-2003 growing season.

Because Amargosa niterwort plants are actively growing in August and September (Hasselquist and Allen 2009; this study) and rhizomes are known to began root growth as early as January (L. DeFalco, personal communication), the growing season for this species was defined as October

–September. Total growing season precipitation values as recorded at the Amargosa Valley CEMP station for the 2002-2003, 2009-2010, and 2011-2012 growing seasons are given in Table 4.

Table 4. Total growing season precipitation as recorded at the Amargosa Valley CEMP station.

Growing Season (September – October)	Total Precipitation (in.)
2002-2003	4.78
2009-2010	3.61
2010-2011	3.67

Monthly precipitation at the Amargosa Valley CEMP station for the three growing seasons is shown in Figure 8, compared to the long-term mean monthly precipitation for each month. Because the Amargosa Valley CEMP station did not begin recording until 1999, there are not enough years of data to compute a reasonable long-term estimate of mean precipitation (at least 30 years are typically required for this). Accordingly, the long-term monthly means shown in Figure 8 come from the National Oceanic and Atmospheric Administration (NOAA) station at Amargosa Farms, Nevada, located only 0.6 km west of the Amargosa Valley CEMP station at an elevation of 2450 ft. (WRCC 2011). These long-term means are based on the period of record for the station, 1965-2010 (data have not yet been input for much of 2011). Figure 9 shows the total growing season precipitation at the Amargosa Valley CEMP station for all of the growing seasons since station began recording in 1999, compared to the mean long-term growing season precipitation as recorded by the Amargosa Farms NOAA station.

In October 2009 eleven piezometers were installed by the Bureau of Land Management with the help of the Amargosa Conservancy and others to monitor the level of groundwater at various locations in the Lower Carson Slough. Figure 10 shows the locations of these piezometers relative to the macroplots used for Amargosa niterwort monitoring. Distance from ground surface to groundwater has been measured monthly at all eleven piezometers by the Amargosa Conservancy.

As Figure 10 shows, Piezometer CS3 is the one most representative of the groundwater beneath the monitored Amargosa niterwort population at Lower Carson Slough. Although other piezometers, particularly CS9, are relatively close to the macroplots used to monitor niterwort, they are not really representative of niterwort habitat.

Figure 11 shows the monthly distances to groundwater at Piezometer CS3 for the 2009-2010 and 2010-2011 growing seasons.

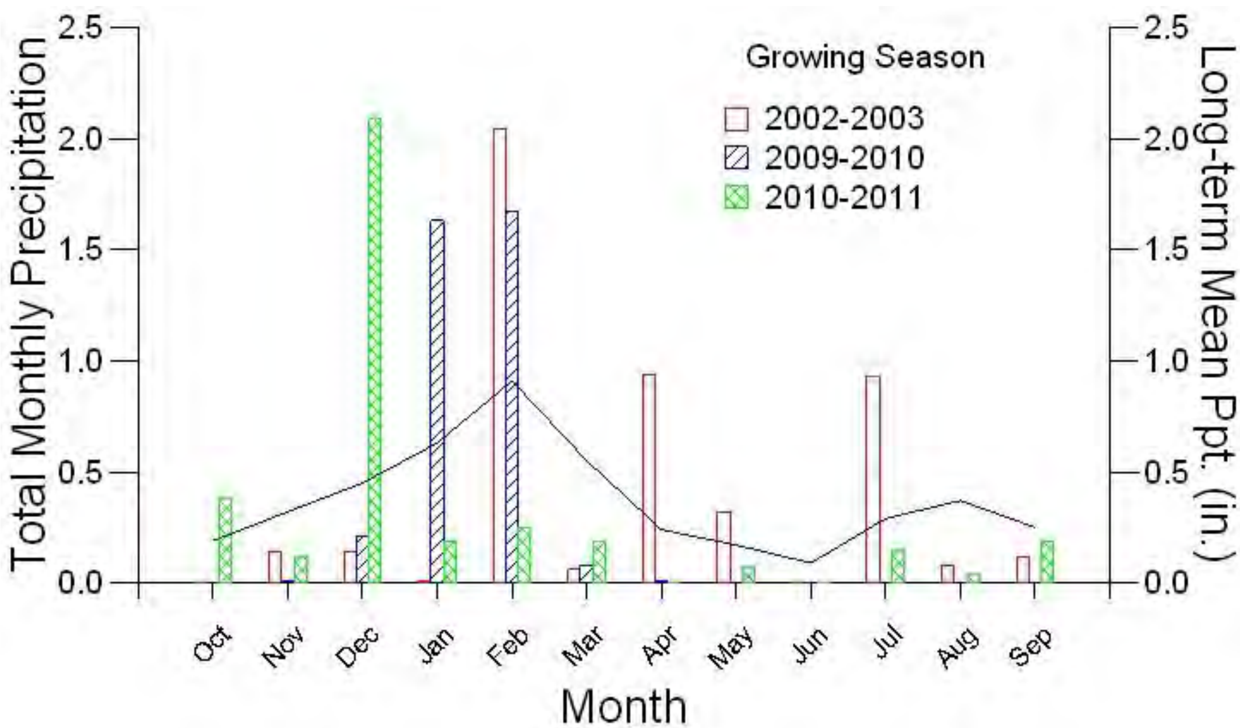


Figure 8. Total monthly precipitation (bars) for the 2002-2003, 2009-2010, and 2010-2011 growing seasons recorded at the Community Environmental Monitoring Program weather station at Amargosa Valley, NV. Also shown is the long-term monthly means (line) from the NOAA weather station at Amargosa Farms, NV (period of record, 1965-2010).

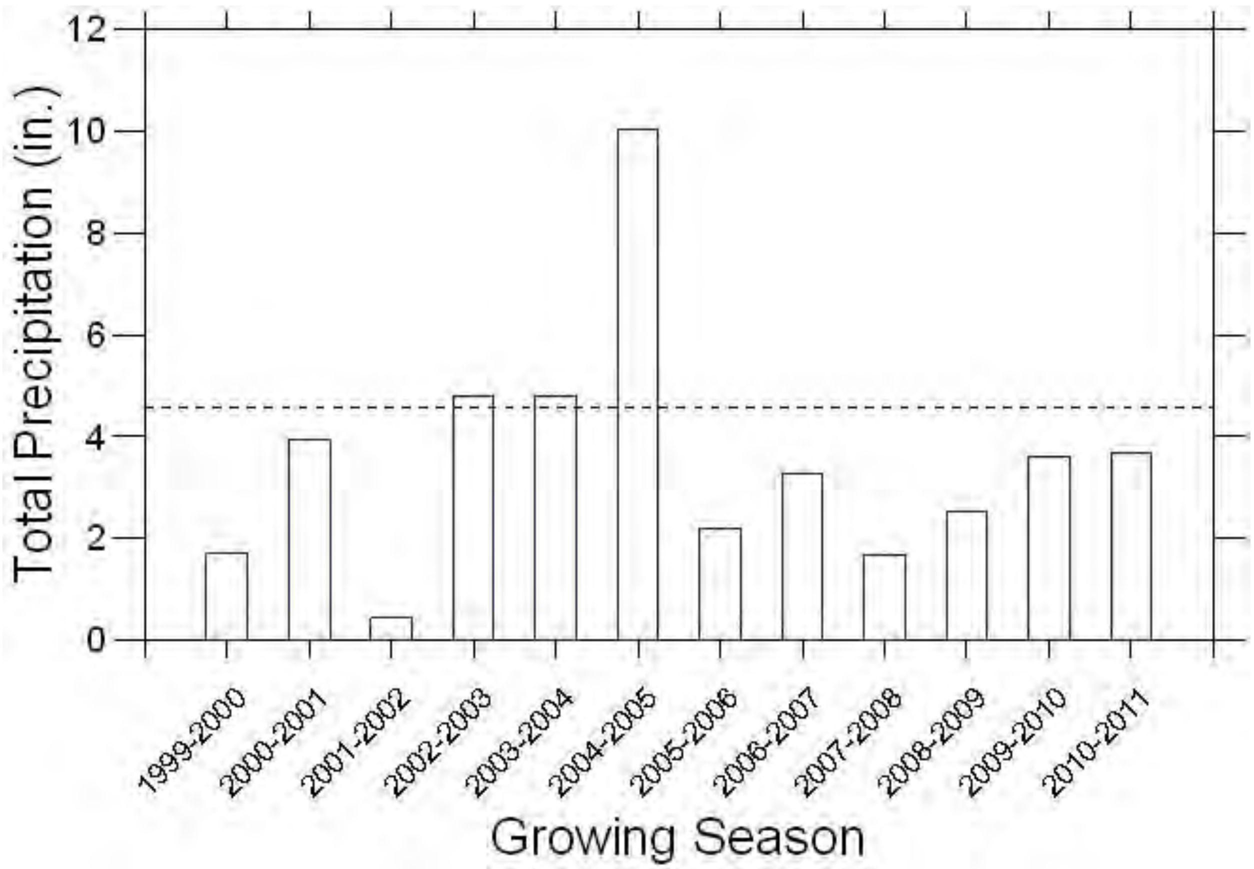


Figure 9. Total growing season (October – September) precipitation at the Amargosa Valley CEMP weather station since the station began recording in 1999. Dashed line is the long-term mean growing season precipitation (4.57 in.) as recorded at the nearby Amargosa Farms NOAA weather station (period of record 1965-2010). Nine of the 12 growing seasons were below the long-term mean total precipitation. The 2004-2005 growing season was more than two times the long-term mean.

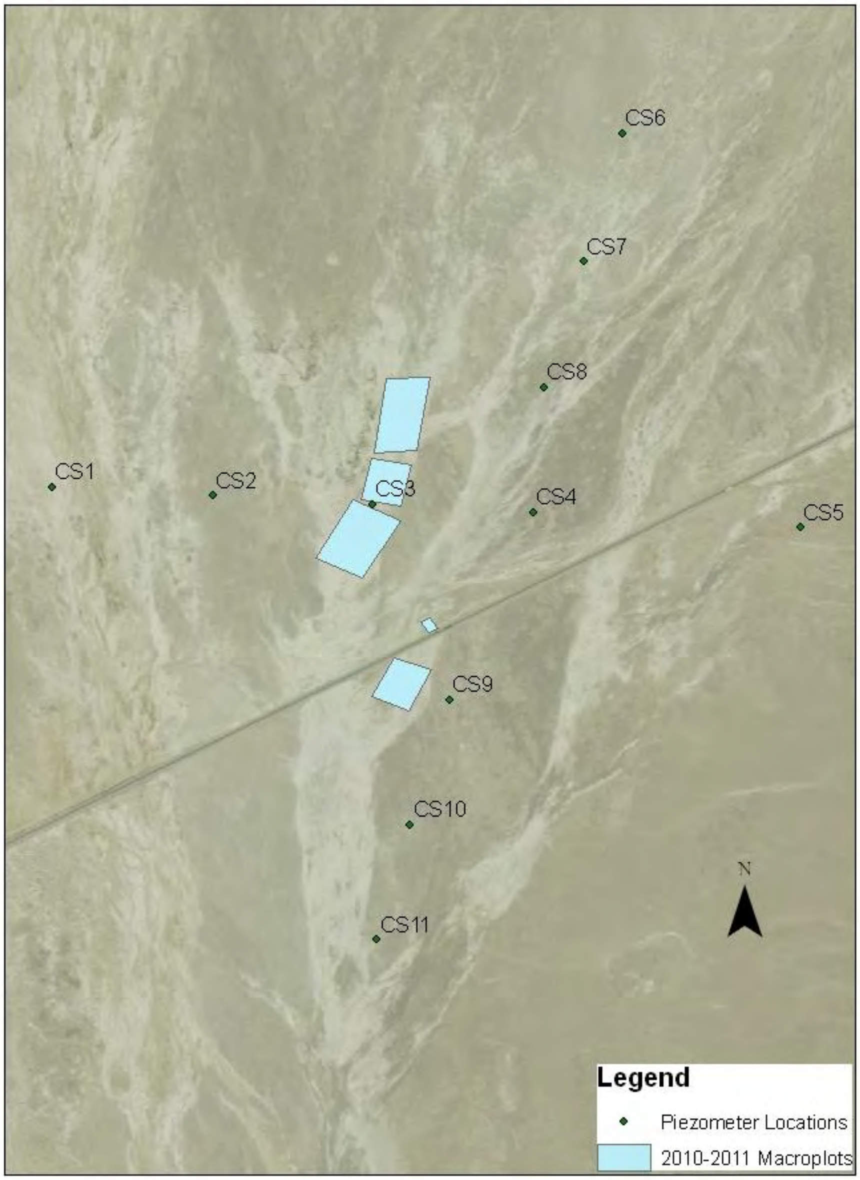


Figure 10. Locations of the 11 Lower Carson Slough piezometers in relation to the 5 macroplots within which Amargosa niterwort is being monitored. Piezometer CS3 occurs immediately adjacent to Macroplots 2 and 3 and is most representative of distance to groundwater in Amargosa niterwort habitat. Although piezometer CS9 is only about 60 m away from Macroplot 5, it is higher in elevation and not representative of niterwort habitat.

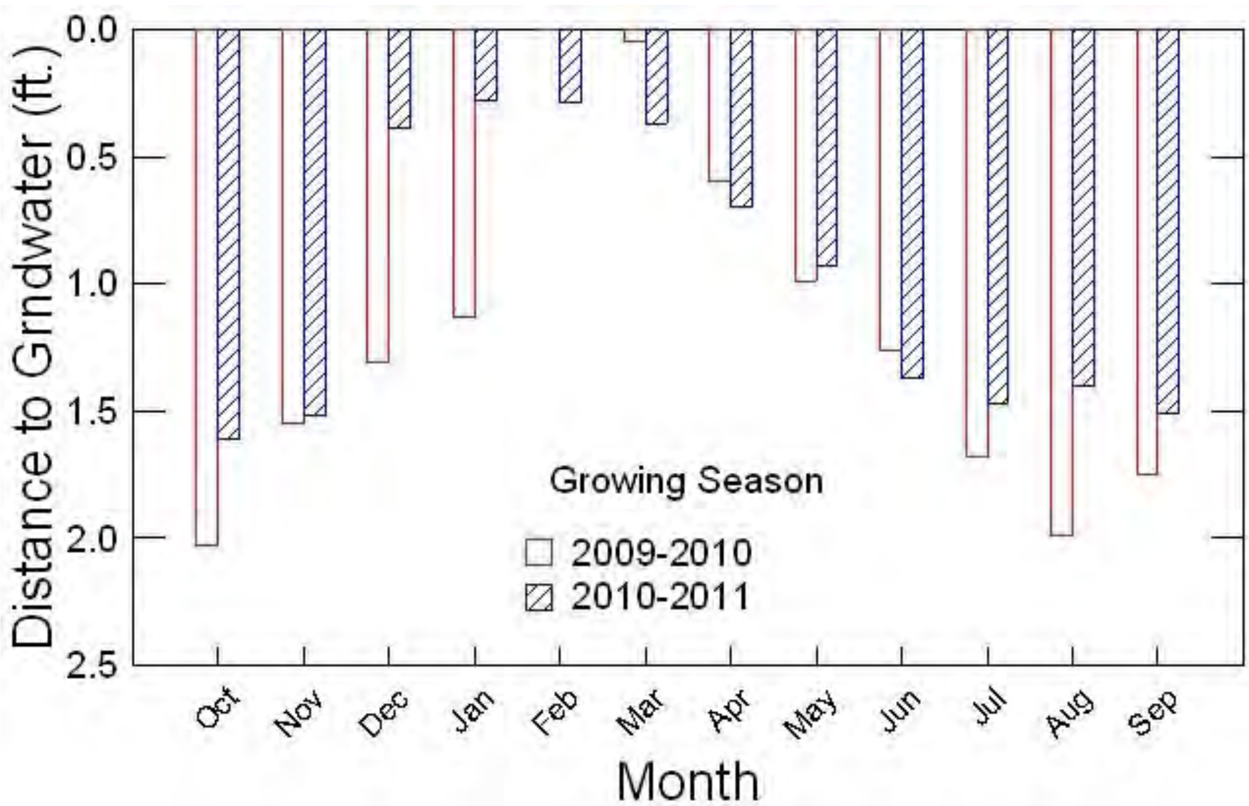


Figure 11. Monthly distances (in feet) to groundwater as measured at Lower Carson Slough Piezometer CS3 for growing seasons 2009-2010 and 2010-2011.

Discussion

Comparison of 2010-2011 and 2003 stem numbers. The Anteon study conducted in 2003 (Johnston and Zink 2004) estimated there were 243,478 (± 69,337) rooted stems in Occurrence 1 (± 95% confidence interval). Occurrence 1 as defined by Anteon is about 4.08 hectares, compared to the combined area of 4.56 hectares of macroplots 1-5. The 2010-2011 estimates for the number of rooted stems in the combined area of macroplots 1-5 were 59,540 (± 24,782) in 2010 and 58,431 (± 21,541) in 2011 (± 95% confidence interval). Thus, based on a comparison of 2003 numbers with 2010-2011 numbers it would appear that Amargosa niterwort has suffered a serious decline since 2003. These numbers, however, cannot be taken at face value. Anteon's 2003 estimate suffers from several problems discussed in Appendix 1. It is quite likely that the Anteon estimate is biased high, based on the nonrandom positioning of at least some Anteon's macroplots in areas with higher niterwort numbers than in Occurrence 1 as a whole. Also, Anteon's confidence interval was calculated incorrectly; a correctly calculated confidence interval (which could not be done with the available information) would likely yield a much wider confidence interval, meaning that the true difference between 2003 and 2010-

2011 may not be nearly as great as first appearances indicate. Finally, as noted earlier there are issues with obtaining accurate counts of rooted stems in quadrats, with the result that not only are comparisons of rooted stems suspect between 2003 and 2010-2011, they are also suspect between 2010 and 2011.

This said, it is quite possible that the numbers of rooted stems and, thus, the overall abundance of Amargosa niterwort was greater in 2003 than in 2010 and 2011, given the magnitude of the difference observed. This may be the result of the higher growing season precipitation experienced during the 2002-2003 growing season (4.78 in., Table 4) compared to the amount of precipitation in the 2009-2010 and 2010-2011 growing seasons (3.61 in. and 3.67 in., respectively, Table 4). As Figure 8 shows, precipitation was far below average in the months October through January in the 2002-2003 growing season, but then much higher than average in February (2.04 in., more than twice the average of 0.91 in.), April (0.94 in., more than three times the average of 0.24 in.), May (0.32 in., almost twice the average of 0.17 in.), and July (0.93 in., more than three times the average of 0.29 in.). Although March, June, August, and September 2003 were far below the average for those months, the high rainfall in February, April, May, and July 2003 may have been more than sufficient to provide water for Amargosa niterwort growth given the very shallow water table at the site (more discussion on the water table below). The much below average rainfall early in the growing season (October – January) may not have much of an impact on growth of niterwort (though it may help to keep groundwater at shallow levels in years in which precipitation after January is low). A reconnaissance visit to Lower Carson Slough in March 2009 revealed only a few very small plants above ground (despite about an inch of rainfall in November 2008), supporting the hypothesis that—at least in some years—the species may not start growth until February or later (although as pointed out earlier, in 2007 U.S. Geological Survey biologists observed root buds on rhizomes as early as January).[6]

Comparison of 2010 and 2011 stem and clump numbers. Estimated numbers of rooted stems were about the same in both 2010 and 2011 (59,540 ± 24,782 in 2010 and 58,431 ± 21,541 in 2011, Table 2). The slightly lower estimate for 2011 was not significant at $\alpha = 0.10$ (paired $t = -0.13$, $P = 0.90$, Table 3). The accuracy problems with counting rooted stems have already been discussed; this attribute will no longer be monitored in future years.

As Table 2 shows, both the estimated number of clumps and frequency were greater in 2011 than in 2010. Clump numbers were estimated to be 33,309 (± 12,895) in 2011, about twice as

[6] Given the fact the plant is actively growing into September, through a summer of very intense heat (high temperatures at the Amargosa Farms NOAA weather station average 104 and 102.2 degrees F, respectively, in July and August), one might suspect that the species would use the C_4 photosynthetic pathway, but Jacobs (2001) found that, based on leaf anatomy and ultrastructure, it is in fact a C_3 species.

many as estimated for 2010 (16,712 ± 5,938), a difference that was significant at $\alpha = 0.10$ (paired $t = 3.40$, $P = 0.002$, Table 3). The 2011 frequency of 0.088 (± 0.027) was significantly greater than the 2010 frequency of 0.061 (± 0.015) at $\alpha = 0.10$ (paired $t = 2.31$, $P = 0.032$, Table 3).

The higher 2011 clump number and frequency cannot be explained by the total growing season precipitation alone because these values are very similar (3.61 in. for 2009-2010 and 3.67 in. for 2010-2011, Table 4). However, the distribution of the precipitation through the growing season was quite different between the two growing seasons. In 2009-2010 there was significant rainfall in January and February (1.63 in. and 1.67 in., respectively, well above the averages for both months), but precipitation was almost nonexistent the remainder of the growing season, from March through September (Figure 8). In 2010-2011 December precipitation was more than four times the average for that month (2.09 in.). The remainder of the growing season, though lower than average for all of the months, at least had some rainfall in every month except April and June (Figure 8). The higher rainfall during the hotter months may at least partially explain the higher clump number and frequency in 2011 compared to 2010.

Groundwater levels recorded at Piezometer CS3 for growing seasons 2009-2010 and 2010-2011 (Figure 11) appear to at least partially follow the pattern of precipitation. For example, groundwater was slightly more than 2 ft. below ground level in October 2009 following a period of very little rainfall since February 2009. Levels remained below 1 ft. until February 2010 when the groundwater reached surface level, presumably in response to high rainfall in January and February 2011 (the rainfall in January fell during the period of January 18-21, after the January groundwater level had already been measured on January 15). The groundwater level remained just slightly below ground surface level in February 2010, after which it steadily declined to 0.6 ft. in April, to 1.0 ft. in May, to 1.3 ft. in June, to 1.7 ft. in July, and to 2.0 ft. in August. It then rose to 1.8 ft. in September, despite the fact that no rain fell between the measurement in August and the one in September. However, a note in the data sheet received from the Amargosa Conservancy, states that the September 2010 measurement marked the first use of a water level sounder to make the measurements, so it is possible that the difference between August and September is an artifact of that change in the method of measurement.

In October 2010 the water level rose from 1.8 ft. below ground to 1.6 ft. below ground, probably as a result of 0.38 in. of rain in early October prior to October's groundwater measurement. The groundwater level then rose slightly to 1.5 ft. below ground in November 2010. The level then rose considerably to 0.4 ft. below ground in December 2010, likely the result of the high rains in December, most of which fell during a large storm December 20 – 22 that dropped 1.78 in. of rain (this was prior to the groundwater measurement which took place on December 31). Although groundwater never reached ground surface level at any time during growing season 2010-2011, it remained less than 0.5 ft. from the surface from December 2010 through March 2011. It then dropped to 0.7 ft. below the surface in April 2011, to 0.9 ft. below the surface in May, and to 1.4 ft. below the surface in June. It remained between 1.4 ft. and 1.5 ft. below the surface for the months of July through September. Note from Figure 11 that groundwater levels in June, July, August, and September of 2011 were closer to the surface than the levels in the same months of 2010 (this difference is particularly evident in August where the groundwater level was 0.5 ft. closer to the surface in 2011 (1.4 ft. below ground surface) than in 2010 (2.0 ft. below ground surface). Thus, higher groundwater levels during the hotter months of 2011 as compared to 2010 may explain the higher Amargosa niterwort frequencies and clump numbers observed in 2011.

Variability in abundance between years. There appears to be considerable year-to-year variability in the abundance of Amargosa niterwort. This variability is exacerbated by the fact that above-ground shoots of the species die and mostly disappear between monitoring episodes, presumably because they become brittle, disconnect from the rhizome and then either blow away later in the summer/fall period or float away during the wet winter months when surface water flows through the slough. A few dried shoots, presumably from the previous year, were observed in both 2010 and 2011, but almost all of the shoots that were present in 2010 were gone in 2011 and replaced by new shoots. This same conclusion was reached by Lesley DeFalco when studying the Amargosa niterwort population below Crystal Reservoir at Ash Meadows National Wildlife Refuge (L. DeFalco, personal communication, November 3, 2011). Figure 12 is a photograph she took of a dead Amargosa niterwort shoot at that population.

Evidence for year-to-year variability comes from the difference in clumps and frequency between 2010 and 2011 (higher numbers in 2011), the likely greater number of stems in 2003 as compared to the 2010-2011 values, and observations by Hasselquist and Allen (2009) during a water use study conducted on Amargosa niterwort in 2005 and 2006. Although they took no measurements of Amargosa niterwort abundance, Hasselquist and Allen noted a "dramatic reduction" in niterwort abundance between 2005 and 2006 as a result of a substantial decrease in the amount of precipitation (growing season 2004-2005 precipitation was more than twice the long-term average, while growing season 2005-2006 precipitation was less than half the long-term average). They believe this decline suggests "the "importance of high surface moisture in early spring for the germination and initial establishment of" Amargosa niterwort. It is unclear whether the new Amargosa niterwort shoots observed each year are the result of germinating seeds, new shoots from underground rhizomes, or both. Under a cooperative agreement with BLM, the Rancho Santa Ana Botanic Garden (RSABG) attempted to collect enough seeds of Amargosa niterwort to serve as a conservation seed collection but were only able to collect 10 viable seeds in 2010 and 51 viable seeds in 2011 (Fraga and Wall 2011). The RSABG report (ibid.) states “This species produces rhizomes and may therefore rely on vegetation propagation as the primary means of reproduction.”

Figure 12. Dead shoot of Amargosa niterwort encrusted in salt, at the population below Crystal Reservoir in the Ash Meadows National Wildlife Refuge. Photograph by Lesley DeFalco, U.S. Geological Survey, Henderson, NV.

In excavating Amargosa niterwort plants for a competition study at the population below Crystal Reservoir, U.S. Geological Service biologists found several shoots that were not connected to a rhizome and that had a shallow taproot. These few plants could were noted as possibly germinated from seed, but such occurrences were rare (L. DeFalco, personal communication, November 3, 2011).

The results of this 2010-2011 study similarly suggest a relationship between Amargosa niterwort abundance and both groundwater levels and the amount and distribution of precipitation on site, but several more years of monitoring Amargosa niterwort, precipitation, and groundwater levels will be required before firmer conclusions are possible on this matter. It does appear, however, that long-term monitoring will be necessary to tease out any effects on niterwort abundance from a lowering of the water table due to groundwater pumping from the year-to-year differences in abundance that result from natural fluctuations in precipitation and groundwater levels.

Note that "abundance" as used here refers to the abundance (clump number, cover, and frequency) that can be observed above ground. It may well be that the below-ground abundance of the species (e.g., number of live rhizomes) may not fluctuate nearly as much as the above-ground expression of the species. It is not possible to monitor below-ground abundance, both because of the difficulty of doing so and the inevitable damage to the species. Above-ground abundance is therefore the only indicator we have of the health of the species.

Use of groundwater by Amargosa niterwort. Hasselquist and Allen (2009), using isotope analysis on water collected from Amargosa niterwort plants during 2005 and 2006, determined that the species uses only what Hasselquist and Allen define as "surface water and soil moisture near the soil surface" in contrast to another endangered plant, Ash Meadows gumplant (*Grindelia fraxino-pratensis*), which uses surface water and soil moisture near the soil surface in early spring but then shifts to using "groundwater" as the soils dry out beginning about the first of May. The authors define "groundwater" as water that is 1 m (3.3 ft.) below the ground surface and "surface water and soil moisture near the ground surface" as water from depths to 30 cm (1.0 ft.) from the ground surface. Although Ash Meadows gumplant occurs in the Carson Slough area it occupies habitats that are more upland than those occupied by Amargosa niterwort. Soils under the gumplant dry out to the point of developing a hardpan layer that precluded Hasslequist and Allen (2009) from collecting surface water samples below 10 cm (0.33 ft.) from the ground surface after June; the same was not true for soils below Amargosa niterwort.

Hasselquist and Allen did not have access to groundwater data immediately adjacent to Amargosa niterwort populations, as the Lower Carson Slough piezometers were not installed until September 2009. The piezometer data seems to support their conclusions that niterwort uses surface water and water close to the ground surface, although their definition of groundwater as water at least 1.0 m below the ground surface does not seem to hold, as groundwater actually reaches the ground surface during some months and years. The water used by Amargosa niterwort seems to either come from the groundwater itself or from groundwater upwelling as suggested by Hasselquist and Allen (2009).

At the Crystal Reservoir population of Amargosa niterwort, U.S. Geological Survey biologists found rhizomes about 8-15 cm (0.3 to 0.5 ft.) below ground, at depths below the 0.5-6 cm (0.02-0.20 ft.) depth where the soil was frozen in January 2007 (L. DeFalco, personal communication, November 3, 2011). Roots were already developed and growing to depths greater than 10-30 cm (0.3-1.0 ft.). Thus, it would appear very possible that the species is using groundwater at depths of 50 cm (1.6 ft.) and possibly deeper.

Future monitoring. Monitoring of Amargosa niterwort will continue annually as long as sufficient funding is available. Future years of monitoring will allow between-year comparisons to be made of the number of clumps, cover, and frequency of the species and allow further observations on the above-ground response of the species to fluctuating rainfall and ground-water levels.

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Appendix 1—Summary of the Anteon Study Conducted in 2003 and Problems with the Study

The Lower Carson Slough Amargosa niterwort occurrence was the target of a previous inventory effort conducted in 2003 by Anteon Corporation under contract with BLM (Johnston and Zink 2004). Based on their observations that the density of niterwort was much greater near the drainage channel running through the alkali flats of Lower Carson Slough, the study's authors delineated two independent sampling areas within which they conducted sampling to estimate population size. The sampling area they identified as Occurrence 1 was in and near the channel, while the other sampling area, Occurrence 2, was farther away from the channel. Figure 1, taken from the 2004 report, depicts the areal extent of these two occurrences.

Despite repeated requests to the junior author of the study (who is a faculty member at San Diego State University; the senior author was a graduate student who is no longer at the University), I was unable to obtain either the raw data from the 2003 field work or the Geographical Information System shapefiles used to create the maps in the report. Therefore, this summary of the report is based solely on the information provided in the report itself.

Study Design

Nine macroplots, each 21m x 30m, were "randomly" positioned within Occurrence 1 (more on issues with respect to the randomness of the positioning process below). Occurrence 2 was also sampled by positioning some number of macroplots of the same size within it. The report is unclear about the actual number of macroplots used in Occurrence 2. The report states in one place (Johnston and Zink 2004, page 17) that 15 macroplots were sampled, but later states (ibid., page 19) that 20 macroplots were sampled. The map in Appendix IV (ibid., page 29) depicts 20 macroplots, but then, in Appendix V (ibid,. page 30), the report gives UTM coordinates for 19 Occurrence 2 macroplots. Because I did not have access to the actual raw data, I was unable to ascertain exactly how many macroplots were sampled.

Macroplots were subsampled with 1m x 3m quadrats, within which numbers of ramets were actually counted. According to Johnston and Zink (2004, page 17), "Rebar was installed at two corners of each macroplot and mapped with a GPS unit for permanent demarcation." Whether the coordinates for both corners of each macroplot were actually recorded is unknown; the report only gives one set of coordinates for each macroplot. It is not clear whether the coordinates given are for one of the corners of each macroplot or for the middle of each macroplot. Nor is the geodetic datum for the macroplot coordinates specified in the report. From visits to Lower Carson Slough and to some of the rebar marking the 2003 macroplots, it appears that the datum for the coordinates given in the report is NAD 27.

Twenty-seven of the possible 210 quadrats were sampled in each of the nine macroplots in Occurrence 1, while 39 quadrats were sampled in each of the 15, 19, or 20 macroplots sampled in Occurrence 2.

The report does not give the dates that the sampling occurred, not even the year. Because the report is dated 10 February 2004, it is assumed the sampling took place sometime during late spring or summer of 2003.

Because *Nitrophila mohavensis* is rhizomatous and several stems can arise from the same rhizome, it is not possible to count genets (genetically distinct individuals). The Johnston and Zink (2004) study therefore focused on counting ramets: "A count of one ramet consisted of a single stem arising from the soil." (ibid., page 15).

Random sampling was not used for Occurrence 1. Nine macroplots, each 21m x 30m, were "randomly" positioned within Occurrence 1 by "blindly throwing a weighted flag into the area and using the landing spot as one corner of each macro-plot." (Johnston and Zink 2004, page 24). Although the authors suggest this method results in a random sample, it in fact does not. The result is a haphazard sample and is biased either consciously or subconsciously. For example, the observer must decide where to stand to throw the flag, a decision that is not random. The boundaries of Occurrence 1 trend generally from north to south, but they take a jog from west to east before heading south again (see Fig. 1). Occurrence 1 spans more than 800m north to south and occurs on both sides of Stateline Road. Given that there is only one macroplot in Occurrence 1 south of Stateline Road, it is apparent that the decision was made to throw only one flag south of Stateline Road. Occurrence 1 south of Stateline Road is about 135m "wide" (west to east). The sole macroplot is located about halfway between the north and south ends of the part of Occurrence 1 south of Stateline Road and within about 15m of the eastern edge of Occurrence 1, leading one to suspect that the observer made a decision to throw the flag from the eastern side of this part of Occurrence 1 somewhere near the middle. This biases the sample toward the middle of this southern occurrence and—probably because it was difficult to throw a weighted flag very far—toward the eastern edge of Occurrence 1.

Occurrence 1 north of Stateline Road is about 575m "long" (north to south) and ranges from about 50m to 80m "wide" (generally west to east). Three of the eight macroplots north of Stateline Road are very close to the eastern boundary of Occurrence 1 and none of the eight macroplots are in the western half of Occurrence 1. Thus, it appears that an observer threw the flag from the eastern side of Occurrence 1 at intervals known only to him/her and that because the flag could not be thrown all the way across Occurrence 1, all eight macroplots are in the eastern half. This clearly biases the sample toward the eastern half of Occurrence 1. An additional issue is that the place within Occurrence 1 that jogs west to east has no macroplot in it.

Many sampling papers and texts warn against haphazard sampling, the category of sampling that throwing a weighted flag falls into (see, for example, Campbell 1989, Lewis 2004, Southwood 1966, Steucek 1986, Schwarz 2009). This would be true even if the flag was thrown from the same location each time (because the force and direction of the throw are conscious decisions of the person throwing the flag). In this case, not only is the force and direction of the throw at the discretion of the observer, but so are the positions from which the observer decides to throw the flag. Although the report says the flag was "blindly" thrown, supposedly to guard against the possible tendency to consciously aim the flag toward areas that have many target plants in them, the other decisions—where to throw the flag from and the direction and force used to throw it, may themselves bias the sample toward areas where more plants are likely to be found. Indeed, when looking at the locations of the macroplots during the 2010 sampling, it appeared that the macroplots were in fact positioned in areas with more plants than many of the other areas within Occurrence 1. In any event, the sample cannot be considered a random sample and the estimate of the population total for Occurrence 1 must be considered biased.

Macroplots were positioned in Occurrence 2 using a procedure that better ensured a truly random sample. A 50m x 50m grid was superimposed on a topographic map and a unique number assigned to each 50m x 50m grid cell. Some number of grid cells (15, 19, or 20, depending on how many were actually sampled) were then randomly selected. The coordinates of the center points of these grid cells were then entered into GPS units for location in the field. The population total estimate for Occurrence 2 can therefore be considered unbiased (though there are definitely problems with the confidence intervals given in the report as discussed below).

Data were not analyzed correctly. Based on the sampling described above, Johnston and Zink (2004, page 19) estimated that there were 243,478 ± 69,337 niterwort ramets in Occurrence 1 and 28,951 ± 20,372 ramets in Occurrence 2. The error terms used in the report (± 69,337 ramets and ± 20,372 ramets) represent 95% confidence intervals. Based on the discussion above, we already know that the Occurrence 1 estimate of 243,478 ramets is biased and likely does not truly represent the real number of ramets in Occurrence 1. But there are also significant problems with the way confidence intervals were calculated.

It is clear from the report that confidence intervals were not calculated correctly. The report (Johnston and Zink 2004, page 19) gives a per quadrat mean of 17.917 and standard deviation of 41.001 for Occurrence 1, which led me to believe that the authors improperly used the quadrat mean and standard deviation to derive a population estimate and confidence interval. To check this I used these summary statistics (mean of 17.917, standard deviation of 41.001) and the area within Occurrence 1 (4.08 hectares) to calculate a population estimate and confidence interval. My calculations (conduced in Microsoft Excel 2007) yielded a population estimate of 243,671 and a 95% confidence interval of ± 70,462, very close to the 243,478 and ± 69,337 calculated by the authors of the report (the differences are likely the result of rounding errors).

Based on these calculations it is clear that for Occurrence 1 the authors incorrectly used the 1m x 3m quadrat standard deviation and sample size to calculate the confidence interval. This approach is incorrect because it assumes that the 243 1m x 3m quadrats sampled in Occurrence 1 are independent random sampling units, something that clearly is not the case given that 27 quadrats were sampled in each macroplot. The design employed by Johnston and Zink is a two-stage sampling design (sensu Cochran 1977; other authors, e.g., Levy and Lemeshow 1999, Lohr 1999, refer to this type of design as two-stage cluster sampling), with macroplots as the primary sampling units and quadrats as the secondary sampling units. Although there are formulas that incorporate standard deviations associated with both the primary and secondary samples, it is the standard deviation of the primary sample that is the most important and, in fact, calculating the confidence interval based only on the primary sample closely approximates the confidence interval calculated using standard deviations from both the primary and secondary samples (Cochran 1977). Because the sample size associated with the primary sample of macroplots is only 9, the resulting standard error will likely be fairly high (unlike the situation when treating the quadrats as the sampling units where the sample size is 243). Although I cannot calculate the correct confidence interval for Occurrence 1 because I do not have access to either the raw data or the macroplot means and standard deviations, I am certain that the true precision of the estimated population size from the 2003 data is much poorer than that given in the report and the actual confidence intervals much wider.

The precision of the estimate in the report is 28.5% (calculated by dividing the 95% confidence interval half-width of 69,337 as given in the report by the report's population estimate of 243,478 and multiplying by 100 to convert the proportion to a percent; the smaller the percent precision the better). It is likely given the very clumped nature of the niterwort population in the Lower Carson Slough that a two-stage sampling design with a primary sample of nine macroplots would yield a confidence interval with a precision greater than 100 percent, at least for a truly random sample.

The confidence interval around the population estimate of Occurrence 2 was most probably calculated in the same, incorrect way as for Occurrence 1: by treating the quadrats as if they were independent sampling units in a one-stage sampling process instead of properly treating the macroplots as the primary sampling units and the quadrats as the secondary sampling units in a two-stage sampling design. Using the Occurrence 2 quadrat summary statistics from the report (quadrat mean of 0.092, quadrat standard deviation of 0.429), I was unable to derive the same population estimate and confidence interval as given in the report. Using those summary statistics and the area given for Occurrence 2 in the report (169.37 hectares) I calculated an estimated population total of 51,940 ramets, compared to the report's estimate of 28,951 ramets. The most likely reason for this discrepancy is that the report's authors used a different area multiplier than the 169.37 hectares given in the report, but without access to the raw data it is not possible to determine this. I calculated a 95% confidence interval of ± 17,023 ramets assuming that 780 quadrats were sampled (20 macroplots x 39 quadrats each), but as we've seen above, the study may have sampled 15 or 19 macroplots and thus fewer quadrats. The report gives a 95% confidence interval of ± 20,372 ramets. Compared to the report's estimated population total of 28,951, this represents a precision of ± 70%, while the precision I calculated from the report's summary statistics was ± 33%. The reason for these discrepancies cannot be determined without access to the raw data collected during the 2003 study.

Conclusion

Before the results of the Anteon report were examined closely, it was hoped that results from monitoring in 2010 could be compared to the results from the 2003 Anteon study to determine if there had been an increase or decrease in the population between those years. If 2010 results could be compared to the 2003 results, a quantitative assessment could be made of Fred Edwards' observations that the Amargosa niterwort had decreased in abundance since 2003. Unfortunately, such a quantitative assessment is not possible. Because of the sampling and analysis issues associated with the Anteon study as discussed above, summary statistics (i.e., estimated population totals and confidence intervals) would not be comparable between the two periods. If one were to ignore the issues associated with the lack of random sampling in Occurrence 1 (the most important occurrence because this is where most of the plants occur), it might be possible to revisit the same nine macroplots sampled by Anteon in 2003 to see if the mean number of plants/macroplot changed between 2003 and 2010. This, however, is not possible without access to either the raw data or the macroplot means and standard deviations, neither of which is provided in the report. A complicating factor is that the macroplots themselves were sampled using 1m x 3m quadrats but the quadrat positions were not marked and the report does not give any information on where the quadrats were positioned inside each of the macroplots. Thus, sampling of macroplots in 2010 would require a new random positioning of quadrats; this would introduce spatial variability that would not be an issue if the same quadrat positions were used in 2010 (i.e., the macroplot values would be different in 2010 even if there had been no change within the quadrats sampled in 2003). This complication by itself is not insurmountable, but the lack of macroplot values makes resampling the macroplots an exercise in futility, particularly given the bias associated with the macroplot selection in 2003.

Appendix 2—Transect Locations

Transect locations. Coordinates are in NAD 83.
Latitude and longitude are in decimal degrees.
Year is the year of transect establishment.
Transect position on baseline is the m mark along the baseline where the transect begins.

Year	Macro plot	Tran sect	Transect Width	Transect Length (m)	Latitude Start	Longitude Start	Latitude End	Longitude End	Transect Position on Baseline (m)	Date of establish-ment	Comments
2010	1	1	0.5	75	36.33089720	-116.36820530	36.33093330	-116.36733580	25	9/10/2010	
2010	1	2	0.5	75	36.33055540	-116.36834590	36.33055396	-116.36750735	63	9/10/2010	
2010	1	3	0.5	75	36.33038037	-116.36844017	36.33036575	-116.36761239	87	9/11/2010	
2010	1	4	0.5	75	36.32998734	-116.36847596	36.33001664	-116.36763723	139	9/11/2010	
2010	1	5	0.5	75	36.32956198	-116.36845457	36.32961060	-116.36762055	198	9/11/2010	
2010	2	6	0.5	75	36.32909915	-116.36865882	36.32901997	-116.36778031	40	9/11/2010	Bush in way so nail back of starting post located at 2.5m instead of 1.0m
2010	2	7	0.5	75	36.32882574	-116.36873648	36.32868792	-116.36786318	72	9/11/2010	
2010	2	8	0.5	75	36.32930029	-116.36862726	36.32918679	-116.36773352	18	9/11/2010	Bush in way so nail back of starting post located at 1.5m instead of 1.0m
2010	2	9	0.5	75	36.32896635	-116.36868794	36.32888337	-116.36780365	50	9/11/2010	
2010	2	10	0.5	75	36.32862633	-116.36873239	36.32849551	-116.36792192	91	9/11/2010	
2010	3	11	0.5	100	36.32857083	-116.36896221	36.32814144	-116.36797205	1	9/12/2010	
2010	3	12	0.5	100	36.32835297	-116.36908392	36.32793167	-116.36810442	29	9/12/2010	
2010	3	13	0.5	100	36.32816974	-116.36922010	36.32774890	-116.36822371	52	9/12/2010	
2010	3	14	0.5	100	36.32793005	-116.36937733	36.32751531	-116.36838394	82	9/12/2010	
2010	3	15	0.5	100	36.32774414	-116.36947243	36.32732774	-116.36849531	102	9/12/2010	
2010	3	16	0.5	100	36.32739408	-116.36972036	36.32697172	-116.36875659	147	9/12/2010	
2010	3	17	0.5	100	36.32806317	-116.36930702	36.32763370	-116.36832590	66	9/12/2010	

Year	Macro plot	Tran sect	Transect Width	Transect Length (m)	Latitude Start	Longitude Start	Latitude End	Longitude End	Transect Position on Baseline (m)	Date of establish-ment	Comments
2010	3	18	0.5	100	36.32789035	-116.36940887	36.32744928	-116.36842648	87	9/13/2010	
2010	5	19	0.5	75	36.32521465	-116.36813019	36.32496747	-116.36735435	8	9/14/2010	
2010	5	20	0.5	75	36.32506053	-116.36820613	36.32472822	-116.36749185	25	9/14/2010	
2010	5	21	0.5	75	36.32488978	-116.36830810	36.32456000	-116.36758891	47	9/14/2010	
2010	5	22	0.5	75	36.32468405	-116.36844073	36.32439041	-116.36768371	73	9/14/2010	
2010	5	23	0.5	75	36.32459358	-116.36849148	36.32425361	-116.36776749	85	9/14/2010	
2011	1	24	0.5	75	36.33096331	-116.36815172	36.33098866	-116.36731215	18	8/28/2011	
2011	1	25	0.5	75	36.33048423	-116.36838516	36.33046530	-116.36753252	73	8/28/2011	
2011	1	26	0.5	75	36.33020661	-116.36844600	36.33019898	-116.36759490	105	8/28/2011	
2011	1	27	0.5	75	36.33010479	-116.36846268	36.33013018	-116.36763559	120	8/29/2011	
2011	1	28	0.5	75	36.32971518	-116.36845681	36.32974898	-116.36761409	168	8/29/2011	
2011	3	29	0.5	100	36.32828154	-116.36914672	36.32789331	-116.36813320	37	8/29/2011	
2011	3	30	0.5	100	36.32758155	-116.36960497	36.32715789	-116.36860737	125	8/29/2011	

Appendix 3—Macroplot Locations

Macroplot coordinates.
Coordinates are in NAD 83.
Latitudes and longitudes are in decimal degrees.

Macroplot	Corner	Latitude	Longitude	Comments
1	NE	36.3311435	-116.3673358	Didn't take corner coordinates--reconstructed from transect coordinates
1	SE	36.3295938	-116.3676206	Didn't take corner coordinates--reconstructed from transect coordinates
1	NW	36.3311105	-116.3682572	Didn't take corner coordinates--reconstructed from transect coordinates
1	SW	36.3295452	-116.3684760	Didn't take corner coordinates--reconstructed from transect coordinates
2	NE	36.3293111	-116.3677335	Didn't take eastern corner coordinates--reconstructed from western coordinates
2	SE	36.3284483	-116.3679722	Didn't take eastern corner coordinates--reconstructed from western coordinates
2	NW	36.3294372	-116.3685245	
2	SW	36.3285680	-116.3688335	
3	NE	36.3281434	-116.3679604	
3	SE	36.3269425	-116.3687520	
3	NW	36.3285880	-116.3689555	
3	SW	36.3273640	-116.3697401	
4	NE	36.3261245	-116.3673485	
4	SE	36.3258975	-116.3671900	
4	NW	36.3260381	-116.3675445	
4	SW	36.3258041	-116.3673772	
5	NE	36.3250416	-116.3673138	
5	SE	36.3241865	-116.3678374	
5	NW	36.3252884	-116.3680908	
5	SW	36.3244700	-116.3685572	

Appendix 4—Data Form Used in 2010

2010 Amargosa niterwort monitoring data sheet

Pilot sampling in 0.5m wide quadrats (belt transects). Stems and clumps whose rooted stems fall on the left edge of the belt are in, those on the left edge are out. Stems and clumps whose rooted stems fall on the bottom edge of each 1m segment are recorded for that segment. Those that fall on the top edge of each segment are recorded in the next segment. Those on the top edge of the last segment are not recorded.

Date:	Observers:	
Macroplot #:	Quadrat #:	Quadrat coordinate:
Quad length:	Quadrat GPS coordinates:	

Segment	# Plants	# Clumps	Segment	# Plants	# Clumps	Segment	# Plants	# Clumps
1			35			69		
2			36			70		
3			37			71		
4			38			72		
5			39			73		
6			40			74		
7			41			75		
8			42			76		
9			43			77		
10			44			78		
11			45			79		
12			46			80		
13			47			81		
14			48			82		
15			49			83		
16			50			84		
17			51			85		
18			52			86		
19			53			87		
20			54			88		
21			55			89		
22			56			90		
23			57			91		
24			58			92		
25			59			93		
26			60			94		
27			61			95		
28			62			96		
29			63			97		
30			64			98		
31			65			99		
32			66			100		
33			67					
34			68					

Appendix 5—Data Forms Used in 2011

Form Used for Transect Sampling in Macroplots 1, 2, 3, and 5

2011 Amargosa Niterwort Monitoring Data Sheet

Counts of rooted stems and clumps and measurement of cover.

Pilot sampling in 0.5m wide belt transects. Stems and clumps whose rooted stems fall on the left edge of the belt are in, those on the right edge are out. Stems and clumps whose rooted stems fall on the bottom edge of each 1m segment are recorded for that segment. Those that fall on the top edge of each segment are recorded in the next segment. Those on the top edge of the last segment are not recorded. Stems are rooted stems--i.e., stems that contact the ground. Branching stems above that are not counted. A clump *for counting purposes ("counting clump")* is a single plant or group of plants separated by at least 2 cm from each other from the place they are rooted. A clump for cover measurements ("*cover clump*") is defined as a plant or group of plants with less than a 2 cm gap *in canopy cover* along the two long dimensions measured. Canopy cover will be calculated from the x and y dimensions measured as part of this study: measure the dimensions of every every single plant or cover clump to the nearest 0.5 cm. Measure the longest extent of each cover clump along each dimension.

Date:	Observers:	
Macroplot #:	Transect #:	Transect x axis coordinate (m point on baseline):
Transect length:	Transect GPS coordinates:	

Segment #	# Clumps	#Stems	Segment #	# Clumps	#Stems	Segment #	# Clumps	#Stems	Segment #	X and Y Dimentions	X and Y Dimentions	X and Y Dimentions
1			35			69						
2			36			70						
3			37			71						
4			38			72						
5			39			73						
6			40			74						
7			41			75						
8			42			76						
9			43			77						
10			44			78						
11			45			79						
12			46			80						
13			47			81						
14			48			82						
15			49			83						
16			50			84						
17			51			85						
18			52			86						
19			53			87						
20			54			88						
21			55			89						
22			56			90						
23			57			91						
24			58			92						
25			59			93						
26			60			94						
27			61			95						
28			62			96						
29			63			97						
30			64			98						
31			65			99						
32			66			100						
33			67									
34			68									

Appendix 5—Data Forms Used in 2011 (cont'd)

Form Used for Conducting Census of Macroplot 4

2011 Data Form for Census of Macroplot 4

Counts of rooted stems and clumps and measurments of x and y dimensions of cover clumps.

Macroplot 4 is 20m by 30m and is gridded into 5m x 5m cells for censusing. Each cell is designated by a letter (see the diagram). The columns, # clumps and # stems, are to be used for counting rooted stems and clumps. Stems are rooted stems--i.e., stems that contact the ground. Branching stems above that are not counted. Clumps are single plants or groups of plants separated by *at least 2 cm from each other from the place they are rooted* (these are called *counting clumps* and are delineated differently from the cover clumps discussed below). In addition to counting clumps and stems you are to measure the x and y coordinates (these measurements are parallel to the edges of the cell) of *cover clumps*. A cover clump is defined as a plant or group of plants with less than a 2 cm gap *in canopy cover* along the two long dimensions measured. Canopy cover will be calculated from the x and y dimensions measured as part of this study: measure the dimensions of every every single plant or cover clump to the nearest 0.5 cm. Measure the longest extent of each cover clump along each dimension. The dimensions columns are to be used for this purpose. As the observer measures the x and y dimensions the recorder will record, for example, "1 x 2.5" or "6 x 8," with the understanding that these measurements are in cm. Whether the dimensions are x and y or y and x is not important--so long as the two measurements are parallel to the edges of the cell.

Note that for counts of stems and counting clumps, only the total cell number for each of these counts will be used in the analysis. Thus, the observer can give whatever numbers s/he considers appropriate to the recorder. We do, however, require separate pairs of dimensions for each cover clump.

A 1 m x 1 m square quadrat is recommended to facilitate counting--25 of these will fit into each 5 m x 5 m cell.

Date:	Observers:
Macroplot #: 4	Cell #:

# Clumps	# Stems	# Clumps	# Stems	Dimensions (cm)	Dimensions (cm)	Dimensions (cm)	Dimensions (cm)